in the opposite chain is covalently linked to a nonproteinaceous polymer molecule, and wherein at least one antibody fragment comprises an antigen binding site that binds to a polypeptide selected from the group consisting of: human vascular endothelial growth factor (VEGF), human p185 receptor-like tyrosine kinase (HER2), human CD20, human CD18, human CD11a, human IgE, human Apo-2 receptor, human tumor necrosis factor-α (TNF-α), human tissue factor (TF), human α4β7 integrin, human GPIIb-IIIa integrin, human epidermal growth factor receptor (EGFR), human CD3, and human interleukin-2 receptor α-chain (TAC).

Remarks/Arguments

The foregoing amendment of claim 1 is fully supported by the specification, for example at page 28, lines 25-28, and does not add new matter.

In connection with the above-identified application, a final Office Action was mailed on May 4, 2001 setting a three-month term for response. The present Preliminary Amendment is filed concurrently with the filing of a Request for Continued Examination, and a request for two months extension of time. Further accompanying the present Amendment is an Associate Power of Attorney and Change of Address letter. The Examiner is respectfully requested to send all future communications to the address shown, to the attention of the undersigned attorney.

Turning now to the Office Action of May 4, 2001, claims 1-7, 9-11, 13, 15, 18-24, and 26-37 are pending in this application, and stand rejected on various grounds.

Prior Rejections

Applicants are pleased to acknowledge the withdrawal of most of the prior rejections, as discussed on page 3 of the present Office Action.

Rejection Maintained

The Examiner maintained the earlier rejection of claims 1-7, 9-11, 13, 15-16, 18-24, and 26-37 (all claims pending) under 35 U.S.C. § 112, first paragraph for alleged lack of enablement on the ground that "the specification fails to teach an example where the disulfide bond linking the cysteine residues in the light or heavy chain is substituted for an amino acid and the cysteine is covalently coupled to a nonproteinaceous polymer that results in a functional antibody."

From this, the Examiner concludes that "undue experimentation would be require[d] to make and use the instantly claimed antibody fragments." With regard to applicants' reliance to McCafferty et al. to show that disulfide bond between the light chain and the heavy chain is not needed for antigen binding, the Examiner notes that "the cited reference is not commensurate in scope with the claims," since the claims are not drawn to scFv antibodies or diabodies as disclosed by McCafferty. The Examiner further cites Haber (Biochemistry 52:1099-1106 (1964)) for its alleged teaching that incorrect interchain disulfide bridge may result in an inactive conformation. Finally, the Examiner cites Tout for its alleged teaching that the removal of the Cys128 residue did not lead to functional assembly of the light (L) chain and heavy (H) chain and stable association between the H and L chains.

Applicants respectfully traverse the rejection.

Contrary to the Examiner's reading, Haber actually teaches that "[t]he formation of an interchain disulfide bridge in an incorrect position prior to complete refolding and reformation of the interchain bridges may result in an altered and inactive conformation." (page 1105). Harber warned the practitioner that the formation of interchain disulfides before proper refolding and reformation of intrachain disulfides (during renaturation of completely denatured antibody chains) could result in an inactive product. The claimed antibody fragment-polymer conjugates do not disrupt the native intrachain disulfide bonds present in the variable or constant domains of the subject antibody fragments. The specification teaches how to synthesize the subject antibody fragments using processes that support native protein folding and intrachain disulfide bond formation. Thus, the problem mentioned by Haber is not applicable to the claimed conjugates.

In addition, Haber teaches that "[i]t has been demonstrated previously that separated, undenatured A and B chains recombine readily to form an active product, even when the interchain disulfide bond is not permitted to reform." (page 1105). Accordingly, not only does the reference fail to support the rejection, Haber actually supports applicants' position that the interchain disulfide bond avoided in the claimed conjugate is not needed for the conjugate to retain the native antigen-binding activity of the parental antibody fragment.

As noted above, Tout was cited as allegedly teaching that the removal of the Cys128 residue did not lead to functional assembly of the L chain and stable association between the H and L chains. The text in Tout referred to in the rejection reads as follows:

> The idea that a stable Fab could be formed by using a truncated H chain CH1 was initiated from a report from Alfthan and coworkers (1), who found that deletion of most of the C-terminal part of the CH1 region except the Cys128H participating in the interchain disulfide bond formation led to a functional assembly with the L chain and formation of the interchain disulfide bond. Therefore, most of the CH1 region is absent, but the interchain disulfide bond between the H and L chains can still form, resulting in a stable association between the two chains. (page 153).

The Office's interpretation of the above-quoted text assumes facts that are no there. Although the relevant text states that an antibody fragment that contains Cys128 is capable of forming a disulfide bond between the L and H chains, resulting in a stable association of the H and L chains, it says nothing about what happens when Cys128 is removed. Nowhere is it indicated in Tout that the removal of Cys128 precludes a stable association between the H and L chains. Since Tout presents no data or discussion relating to an antibody fragment lacking Cys128, Tout fails to support the Examiner's interpretation. In fact, Haber teaches that the absence of an interchain disulfide bond between the H and L chains does not destroy stable association of the H and L chains or antigen-binding activity.

In view of the foregoing arguments, the Examiner is respectfully requested to reconsider and withdraw the present rejection.

New Rejection

Claims 1-7, 9-11, 13, 15-16, 18-24, and 26-37 were rejected under 35 U.S.C. § 112, second paragraph for reciting "portion of a parental antibody." The Examiner questioned whether this meant a heavy and light chain and an amino acid residue, or a light and heavy chain and an Fc region, etc.

Claim 1 as currently amended is clear in that the heavy chain portion is free of the heavy chain constant domains of the Fc region. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

The Information Disclosure Statement

Applicants note that the Examiner has no access to copies of the references cited in the PTO-1449 form of record from the parent file, and are in the process of copying and assembling the documents. Copies of the references will be filed shortly.

It is believed that all claims pending in this application are *prima facie* condition of allowance. Applicants respectfully request that a timely Notice of Allowance be issued in this case, after the Examiner will have received and reviewed the references to be submitted.

Attached hereto is a marked-up version of the foregoing claim amendment. The attached page is captioned "**Version with markings to show changes made.**"

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: October 4, 2001

By: _____

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Version with markings to show changes made

In the Claims:

Claim 1 has been amended as follows:

1. (Twice amended) A conjugate consisting essentially of at least one antibody fragment covalently attached to no more than about 2 nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD, wherein the antibody fragment comprises a heavy chain and a light chain corresponding to a heavy chain and a light chain portion of a parental antibody, wherein said heavy chain portion is free of the heavy chain constant domains of the Fc region, and wherein in the portion of the parental antibody the heavy and light chains are covalently linked by a disulfide bond between a cysteine residue in the light chain and a cysteine residue in the heavy chain, wherein in the antibody fragment the cysteine residue in the light or heavy chain is substituted with another amino acid and the cysteine residue in the opposite chain is covalently linked to a nonproteinaceous polymer molecule, and wherein at least one antibody fragment comprises an antigen binding site that binds to a polypeptide selected from the group consisting of: human vascular endothelial growth factor (VEGF), human p185 receptor-like tyrosine kinase (HER2), human CD20, human CD18, human CD11a, human IgE, human Apo-2 receptor, human tumor necrosis factor-α (TNF-α), human tissue factor (TF), human α4β7 integrin, human GPIIb-IIIa integrin, human epidermal growth factor receptor (EGFR), human CD3, and human interleukin-2 receptor α-chain (TAC).